

ARCHAEBACTERIAL RHODOPSIN SEQUENCES: IMPLICATIONS FOR EVOLUTION

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It has been proposed over 10 years ago that the archaebacteria represent a separate kingdom which diverged very early from the eubacteria and eukaryotes. It follows, therefore, that investigations of archaebacterial characteristics might reveal features of early evolution. Yet, the phylogeny of the archaebacteria and its constituent groups is still subject to controversy. Few archaebacterial genes which code for proteins, and could begin to help in deciding these important issues, have been sequenced so far. The bacterial rhodopsins are found in halophilic archaebacteria, and constitute a relatively well characterized group of membrane proteins. So far, two genes, one for bacteriorhodopsin and another for halorhodopsin, both from *Halobacterium halobium*, have been sequenced. We cloned and sequenced the gene coding for the polypeptide of another one of these rhodopsins, a halorhodopsin in *Natronobacterium pharaonis* (named here pharaonis halorhodopsin). Peptide sequencing of cyanogen bromide fragments, and immuno-reactions of the protein and synthetic peptides derived from the C-terminal gene sequence, confirmed that the open reading frame was the structural gene for the pharaonis halorhodopsin polypeptide. The flanking DNA sequences of this gene, as well as those of other bacterial rhodopsins, were compared to previously proposed archaebacterial consensus sequences. In pairwise comparisons of the open reading frame with DNA sequences for bacterio-opsin and halo-opsin from *Halobacterium halobium*, silent divergences (mutations/nucleotide at codon positions which do not result in amino acid changes) were calculated. These indicate very considerable evolutionary distance between each pair of genes, even in the same organism. In spite of this, the three protein sequences show extensive similarities, indicating strong selective pressures.